FUNCTIONAL CHARACTERISATION OF A NOVEL IMMUNE MODULATORY MOLECULE FROM *FASCIOLA HEPATICA*

by

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CERTIFICATE OF AUTHORSHIP/ORIGINALITY

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

Signature of Student: _____

Date: 22nd December, 2014

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Journal Publications

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Raquel Alvarado, Bronwyn O'Brien, Akane Tanaka, John P. Dalton, Sheila Donnelly, 2014; A parasitic helminth-derived peptide that targets the macrophage lysosome is a novel therapeutic option for autoimmune disease. Immunobiology. (In press, DOI 10.1016/j.imbio.2014.11.008).

Raquel Alvarado, Joyce To, Anita Pinar, Ashley Mansell, Mark Robinson, Bronwyn O'Brien, John Dalton, Sheila Donnelly, 2014; The *F. hepatica* cathelicidin like peptide (FhHDM-1) modulates the activation of the NLRP3 inflammasome in macrophages. (Manuscript in preparation).

Conference presentations

Raquel Alvarado, Maria Lund, Mark W. Robinson, Andrew Hutchinson, Joyce To, John P. Dalton, Bronwyn O'Brien and Sheila Donnelly, 2012; Presentation entitled: A novel molecule secreted by *Fasciola hepatica* modulates the activity of innate immune cells. 42nd Annual Scientific Meeting of the Australasian Society for Immunology.

Raquel Alvarado, Joyce To, Maria Lund, Mark W. Robinson, Andrew Hutchinson, Bronwyn O'Brien, John P. Dalton and Sheila Donnelly, 2013; Presentation entitled: A novel molecule secreted by the parasite *F. hepatica* modulates the response of macrophage NLRP3 inflammasome. 27th Annual Conference of the European Macrophage & Dendritic Cell Society.

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ABBREVIATIONS

ACEC	Animal Care and Ethic Committee
ADCC	Antibody dependent cell mediated cytotoxicity
AIM2	Absent in melanoma 2
ALUM	Aluminium salts
AMPs	Antimicrobial peptides
APC(s)	Antigen presenting cell(s)
ARC	Animal Resources Centre
ASC	Apoptosis associated speck like protein
ATCC	American Type Culture Collection
Az	Azide
BcR	B cell receptor
BMDMs	Bone marrow derived macrophages
BSA	Bovine serum albumin
CAT	Catalases
Cav-1	Caveolin-1
ChTx	Cholera toxin subunit B
CLIC	Clathrin-independent non-caveolar pathway
CO_2	Carbon dioxide
DAMPs	Damage-associated molecular patterns
DAPI	4'6-diamidino-2 phenylindole, dilactate
DMSO	Dimethyl sulfoxide
DC(s)	Dendritic cell(s)
E. coli	*Escherichia coli*
ELISA	Enzyme-linked immunosorbent assay
ES	Excretory secretory products
FACS	Fluorescence-activated cell sorting (flow cytometry)
FBS	Foetal bovine serum
FhCL1	*Fasciola hepatica* cathepsin L-1
FhES	*Fasciola hepatica* excretory/secretory products
FhHDM-1	*Fasciola hepatica* helminth defence molecule-1
FhHDM-1p2	FhHDM-1 peptide 2
FhPrx	*Fasciola hepatica* peroxiredoxin

Geo Mean	Geometric mean
GPx	Glutathione peroxidase
hCAP18	Human cationic antimicrobial protein 18kDa
HDMs	Helminth defence molecules
HDPs	Host defence peptides
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HVS	*Herpesvirus saimiri*
IAPP	Islet amyloid polypeptide
IFNγ	Interferon gamma
Ig	Immunoglobulin
IL	Interleukin
IMDM	Iscove's modified Dulbecco's medium
IPSE	IL-4 inducing principle of schistosome eggs
kDa	Kilo daltons
LBP	LPS-binding protein
LNFPIII	Lacto-N-fucopentaose III
LPS	Lipopolysaccharide
MAPKs	Mitogen-activated protein kinases
MCD	Methyl-β-cyclodextrin
M-CSF	Macrophage colony stimulating factor
MHC	Major histocompatibility complex
MIC	Minimal concentration capable of inhibiting visible microbial growth
MIF	Migration inhibitory factor
MPR	Mannose phosphate receptor
mRNA	Messenger ribonucleic acid
MS	Multiple sclerosis
MSU	Mono sodium urate
MW	Molecular weight
MyD88	Myeloid differentiation factor 88
N/A	Not applicable
Na_2CO_3	Sodium carbonate
Nano-SiO_2	Silicon dioxide (nanoparticles)
NH_4Cl	Ammonium chloride
NPG	N-propyl gallate microscopy mounting media

NLRs	NACHT-leucine-rich repeat receptors
(NOD)-like receptors	Nucleotide-binding oligomerisation domain protein like receptors
OD	Optical density
O/N	Overnight
OPep	Ovalbumin peptide
Ova	Ovalbumin
PAMPS	Pathogen-associated molecular patterns
PBMCs	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PC	Phosphorylcholine
PFA	Paraformaldehyde
PGE_2	Prostaglandin E2
PI	Peak I
PII	Peak II
PI 3-K	Phosphoinositide 3 kinase
Prx	Peroxiredoxin
RecFhHDM-1	Recombinant *Fasciola hepatica* helminth defence molecule 1
RELM-α	Resistin-like molecule-alpha
RIPA	Radioimmunoprecipitation Assay Buffer
ROS	Reactive oxygen species
RPMI	Roswell Park Memorial Institute 1640 medium
RP-HPLC	Reversed-phase high performance liquid chromatography
RT	Room temperature
SmCB1	*Schistosoma mansoni* cathepsin B
SDS-PAGE	Sodium dodecyl sulphate- polyacrylamide gel electrophoresis
SEA	Soluble egg antigens
SEMs	Standard errors of the means
sFhHDM-1	Synthetic *Fasciola hepatica* helminth defence molecule-1
SiO_2	Silicon dioxide (nanoparticles)
SOD	Superoxide dismutases
T1D	Type I diabetes
TBS	Tris buffer saline
TcR	T cell receptor
TGF-β	Transforming growth factor beta

TGN	Trans-Golgi network
Th	T helper cells
Tip	Tyrosine kinase interacting protein
TLR	Toll like receptor
TNF	Tumour necrosis factor
TMB	3,3′,5,5′-Tetramethylbenzidine liquid substrate system for ELISA
Treg	T regulatory cells
TRIF	TIR domain-containing adaptor inducing IFN-β
TX100	Triton X 100
vATPase	Vacuolar adenosine triphosphatase
v/v	Volume / volume
w/v	Weight / volume

ABSTRACT

The ability of tissue dwelling helminth parasites to induce chronic long term infections, is enabled by the establishment of T helper 2/ regulatory T cell (Th2/Treg) immune responses within their mammalian hosts. Such responses prevent the expulsion of the parasites, whilst simultaneously avoiding excessive inflammation/fibrosis arising within the host, as a consequence of tissue damage induced by helminth migration. Importantly, helminths excrete and secrete a series of molecules (collectively known as ES products), which not only play major roles in parasite biology, but also exert direct immune modulatory functions, promoting the establishment of Th2/Treg immunity. The trematode, *Fasciola hepatica*, is an excellent model of helminth-mediated immune modulation, because it induces a very rapid switch towards Th2 responses in its mammalian hosts and inhibits Th1 immunity. Fractionation of the ES products of *F. hepatica* has identified three major immune modulatory components: the protease cathepsin L1, the antioxidant peroxiredoxin, and a previously uncharacterised peptide, FhHDM-1.

Structural analysis of FhHDM-1 revealed a close resemblance to the cathelicidin, LL-37, a well characterised mammalian immune-modulating peptide. Therefore, a putative immune modulatory role for FhHDM-1 was explored in this project. Immunofluorescent confocal microscopy demonstrated that FhHDM-1 interacted with macrophage lipid rafts, prior to being actively internalised by cholesterol- and cytoskeletal network-dependent endocytosis, with progressive compartmentalisation of the peptide into early endosomes and endolysosomal vesicles. Flow cytometry studies indicated that, once internalised, FhHDM-1 enhanced the rate of endocytosis of dextran by macrophages. Despite this, FhHDM-1 was found to impair the acidification of macrophage endolysosomes and as a consequence, the efficient processing and subsequent presentation of ovalbumin to T cells was prevented, as assessed by decreased detection of digested fluorescent ovalbumin and reduced IL-2 secretion by transgenic $CD4^+$ T cells. Additionally, FhHDM-1 impaired NLRP3 inflammasome activation by lysosomal disruptive agents in macrophages. This was found to be a consequence of reduced cathepsin B activity (due to FhHDM-1 induced suboptimal lysosomal acidification), which was incapable of stimulating inflammasome complex formation, thus avoiding IL-1β and caspase-1 cleavage.

These findings suggest that by targeting endolysosomal activity, FhHDM-1 limits macrophage function. Therefore, the current study is the first to demonstrate that FhHDM-1 possesses immune modulatory properties, which are directed by a mechanism not previously described for a helminth-secreted cathelicidin-like peptide.